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Research Article

Occurrence and antibiotic susceptibility of *Streptococcus pyogenes* isolated from throat of patients that attended federal medical centre Umuahia, Abia state

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A total of 24 throat samples were collected from Patients in Federal Medical Centre Umuahia, Abia State, to evaluate the prevalence of *S. pyogenes* and its antibiotic sensitivity. 17(70.8%) samples yielded *Streptococcus pyogenes* which was identified following some identification test. The incident rate was higher among those within the age of 5-25 years (53%). 58% of the isolate were from females. *S. pyogenens* showed 100% sensitivity to levofloxacin, vancomycin, penicillin G and amoxicillin and was resistant to tetracycline (58.8%). Penicillin, amoxicillin, levofloxacin and vancomycin could serve at first line drug of choice for the treatment of *S. pyogenes* infection.

Key words: Occurrence, resistance, Streptococcus pyogenes, Umuahia

INTRODUCTION

Streptococcus pyogenes is estimated to be present in 5-15% of normal individual in the respiratory track, skin and anus without any sign of disease. The pathogenesis of *Streptococcus pyogenes* is mediated by a variety of factors, one of them is streptolysin 'O' toxin which damage cell membrane and account for the haemolysis demonstrated on sheep blood agar (Ozturk et al., 2004)
Disease spectrum of *Streptococcus pyogenes* ranges from mild infection as pharyngitis, tonsilities and impetigo to life threatening infection like necrotizing fascititis and toxic shock-like syndrome. These are often followed by post infective sequelae of rheumatic fever, rheumatic heart disease and post streptococcal acute glomerulonephritis (Capoor et al*;* 2006). Severe forms of this disease have been detected in various part of the world (Stevens et al*;* 1994).
Streptococcus pyogenes infection is ordinarily spread by direct person-to-person contact most likely via drops of saliva, nasal secretion, contaminated fingers, dust or formites (Arguelles et al*;* 2004).

Streptococcus pyogenes is one of the most virulent species of Streptococcus causing human infections (Euzeby et al., 2012). *S. pyogenes* is a prototype bacterium that causes exotoxins-mediated infections, it produces a plethora of exotoxins, super-antigens and cell wall associated proteins resulting in diverse clinical manifestations, ranging from classical pyogenic infections to toxic shock syndrome and post-infection immune mediated sequelae. Streptococal pyoderma and pharyngotonsillitis remain common infection with a heavy global burden of disease (Carapetis et al*;* 2005).

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S. pyogenes is considered to be a human-adapted pathogen. *S. pyogenes* is the Streptococcal species most commonly associated with epidemic and outbreaks are characterized by the diversity of clinical syndromes and occurrence in both the community (such as within families, schools, day care center and prisons) and health care institutions. However, during the latest 2009 pandemic influenza, *S. pyogenes* has re-emerged as a cause of bacterial super infection.

All Group A streptococcus are sensitive to penicillin G, and most are sensitive to erythromycin.

A high frequency of resistance to erythromycin in *Streptococcus pyogenes* has been reported particularly in countries where antibiotics are over used. In Nigeria there is no existing control program for Streptococci, more over such programs are not in the row. The increase in the incidence of strains that are resistant to current antibacterial agents highlights the need to assess the occurrence and resistance of this pathogen to antibiotics.

MATERIALS AND METHODS

Sample Collection

Throat swabs of 24 patients within the age of 5-67 years were collected using sterile throat swab sticks. The swab sticks were rubbed with rotation over tonsilar area, then the arch of area and finally the posterior pharyngeal wall. Each sample was labeled with various information including age and sex. The samples were transported to the laboratory in sterile condition within 1-2 hours for processing.

Sample Processing

The sample swab was rubbed by rotating over a large area about one third of the surface on a blood agar plate and was streaked out with a loop over the remaining part of the plate and incubated at 37^0C for 24 hours. *Streptococcus pyogenes* produced beta-hemolytic colonies (that is the colonies were surrounded by zone of complete haemolysis with decolorization of haemoglobin). The suspected colony was taken out from primary culture on blood plates and subcultured on crystal violet blood agar(CVBA) plate by placing a bacitracin disc (0.05 units) over initial streaked area and cotrimoxazole disc in secondary streaked area and incubated at 37^0C for 24 hours. A haemolytic colonies slowing bacitracin sensitive and cotrimoxazole resistivity on CVBA was further identified by Gram staining, catalase test and Pyrrolidonyl Arylamisase (PYR) test.

Antibiotic Susceptibility Test

All the identified Group A streptococci isolate (*S. pyogenes*) from throat were subjected to *in-vitro* susceptibility test my modified Kirby-bauer disc diffusion method. The antibiotic used in the study were; Teracycline (30µg), Erythromycin (15µg), Cephalexin (30µg), Penicillin G. (10µg), Gentamycin (10µg), Ciprofloxacin (10µg), Amoxicillin (30µg), Levofloxacin (20µg), Chlorophenicol (10µg) and Vancomycin (10µg). Mueller-Hinton agar plates were inoculated with the isolates antibiotics discs were placed on the medium and plates were incubated at 37^0C for 18-24 hours and results were obtained by measuring the diameter of the zones of inhibition.

RESULTS

Among 24 patients, 11 (45.8%) were males and 13 (54.2%) were females. Out of 24 throat swabs studied, *S. pyogenes* was isolated from 17 samples (70.8%). 7(41.2%)of then isolates were from males whereas 10(58.8%) were from females. Highest colonization of *S. pyogenes* was found in the age group. 5-25 years, followed by 47-67years and lowest among age group 26-46 years (Table 2).

S. pyogenes isolates showed a relative high rate of resistance towards tetracycline (58.8%) followed by Erythromycin (17.7%). The isolates showed 100% sensitivity to levofloxacin, vancomycin, penicillin G and amoxicillin (Table 3).

DISCUSSION

Group A beta-hemolytic streptococci (GABAS) is among the most prevalent bacterial infection and constitute 60%-80% of all cases of exudates pharyngitis (Rijal et al*.,* 2009).Out of 24 samples, age groups ranging from 5-67 years, *S. pyogenes* was isolated from 17 samples (Table 1) 10 (58.8%) were females and 7 (41.2%) from males. High occurrence of *S. pyogenes* in females than males could be as a result of influence of female hormones on adherence ability of *Streptococcus pyogenes* strains to the host cell. However it is in disagreement with the results obtained in turkey as reported by Durmaz et al*.,* (2003). In relation to age, the condition was most prevalent in the age between 5-25 years, the highest prevalence occurring in 7 years old children (schoolchildren) as a result of the close proximity of a large number of susceptible children in a relatively contained environment; such as classrooms and churches, as the disease spread through person to person contact, through nasal droplets, secretions or saliva and spread when those secretions come in contact with the mouth, nose, eye, scrap and wound. The condition was also seen to be high in age group 47-67due to the fact that most of them are immune-compromised patients with diabetes mellitus. This is in

Table 1. Colonial morphology and biochemical reaction of isolates

Gender	Colony Morphology	Gram Staining (GT)	Catalase Test (CT)	Pyrrolidonyl Test (PYR)	Possible Organisms
	S_1 Colourless, Mucoid	+	-	+	*S. pyogenes*
	S_4 Colourless Mucoid	+	-	+	*S. pyogenes*
	S_5 Translucent Shiny	+	-	-	*S. Pneumoniae*
Male	S_7 Colourless Mucoid	+	-	+	*S. pyogenes*
	S_9 Colourless Mucoid	+	-	+	*S. pyogenes*
	S_{10} Grey white Mucoid	-			*K. Pneumoniae*
	S_{13} Colourless Mucoid	+	-	+	*S. pyogenes*
	S_{17} Grey white Mucoid	-			*K. Pneumoniae*
	S_{18} Grey white Mucoid	-			*K. Pneumoniae*
	S_{21} Colourless Mucoid	+	-	+	*S. pyogenes*
	S_{24} Colourless Mucoid	+	-	+	*S. pyogenes*
	S_2 Colourless, Mucoid	+	-	+	*S. pyogenes*
	S_3 Colourless Mucoid	+	-	+	*S. pyogenes*
	S_6 Colourless Mucoid	+	-	+	*S. pyogenes*
Female	S_8 Colourless Mucoid	+	-	+	*S. pyogenes*
	S_{11} Grey white Mucoid	-			*K. Pneumoniae*
	S_{12} Colourless Mucoid	+	-	+	*S. pyogenes*
	S_{14} Colourless Mucoid	+	+		*S.pyogenes*
	S_{15} Colourless Mucoid	+	-	+	*S. pyogenes*
	S_{16} Colourless Mucoid	+	-	+	*S. pyogenes*
	S_{19} Grey white Mucoid	-			*K. Pneumoniae*
	S_{20} Colourless Mucoid	+	-	+	*S. pyogenes*
	S_{22} Colourless Mucoid	+	-	+	*S. pyogenes*
	S_{23} Colourless Mucoid	+	-	+	*S. pyogenes*

Total Number of Male =11; Percentage Number of Male = 45.8%
Total Number of Female = 13; Percentage Number of Female = 54.2%
Key:S = sample

Table 2. Frequency of Occurrence of *S. pyogenes* in Relation to Sex and Age

Sex	Age	No. of Throat Swabs	No. of samples positive	Percentage
	5 – 25	4	3	75
Male	26-46	2	1	50
	47-67	5	3	60
	Total	**11**	**7**	**41.2**
Female	5 – 25	6	6	100
	26 - 46	1	1	50
	47 - 67	5	3	60
	Total	**13**	**10**	**58.8**
	Gross Total	**24**	**10**	**77**

Table 3. Antibiotic Susceptible patterns of *S. pyogenes* Isolates from throat swabs

Antibiotics	Total isolate	Sensitive		Intermediate		Resistant	
		N	%	N	%	N	%
Levofloxacin		17	100	-	-	-	-
Ciprofloxacin		13	76	4	24	-	-
Vancomycin		17	100	-	-	-	-
Penicillin G.		17	100	-	-	-	-
Gentamycin	17	14	82	3	18	-	-
Amoxicillin		17	100	-	-	-	-
Erythromycin		9	52	5	29.4	3	17.7
Chloramphenicol		15	88.2	2	11.8	-	-
Tetracycline		6	35	1	5.9	10	58.8
Cephalexin		14	82.4	3	17.6	-	-

agreement with the study carried out in Pokhara as reported by Rijal et al*.,* (2003). None of the 17 isolates was resistant to penicillin, vancomycin, amoxicillicn and levofloxacin, while slight resistance of *S. pyogenes* was observed with ciprofloxacin, gentamycin, chloromphenicol and cephalexin; this corresponds with the results obtained in USA with 21% resistance as reported by Guege et al*.,* (2009). *S. pyogenes* showed almost completely resistant to tetracycline and erythromycin. The resistance could be due to the presence of mef (A), tet (O), tet(M) and erm (A) genes in *S. pyogenes.* Giovanetti et al*.,* (2004)

CONCLUSION

Acute respiratory tract infections despite antimicrobial therapies remain frequent and a real health problem. *S. pyogenes* show great variability overtime in incidence and clinical presentation. Infections with *S. pyogenes* are currently among the main cause of mortality in developing countries. The diversity and severity of its clinical spectrum should encourage clinicians and microbiologist to pay more attention, it is important to always identify and explore susceptibility of these pathogens to antibiotics in order to contribute to the improvement of care. Result obtained shows that all strains of *S. pyogenes* were sensitive to penicillin, amoxicillin, levofloxacin and vancomycin thus remain the first choice for the treatment of *S. pyogenes* infection.

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